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Lack of association between interleukin-1 gene polymorphism and prognosis in severe traumatic brain injury patients

Ausência de associação entre polimorfismo do gene da interleucina-1 beta e o prognóstico de pacientes com traumatismo crânio-encefálico grave

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ABSTRACT

Objective: Traumatic brain injury is the major cause of death among individuals between 1-45 years-old. The outcome of traumatic brain injury may be related to brain susceptibility to the injury and genetic factors. Genes that may affect traumatic brain injury outcome are being investigated, however there is still few data concerning the association between genetic polymorphisms and traumatic brain injury outcome. The interleukin-1 beta gene (*IL-1B*) is one of the most studied genes, because levels of this cytokine are increased after traumatic brain injury and this can worsen prognosis. The aim of this study was to test whether the -31C/T polymorphism, located at the promoter region of the *IL-1B* gene, is associated with primary short-term outcome (death or intensive care unit discharge) in severe traumatic brain injury patients.

Methods: Were studied 69 patients admitted with severe traumatic brain injury in three hospitals of the metropolitan region of Porto Alegre. The polymorphism was analyzed by polymerase chain reaction, followed by restriction digestion.

Results: Severe traumatic brain injury was associated with a 45% mortality rate. No significant differences were observed in the allele and genotype frequencies between patients stratified by traumatic brain injury outcome.

Conclusion: Our findings suggest that -31C/T IL-1B gene polymorphism have no significant impact on the outcome of patients after acute severe traumatic brain injury.

Keywords: Interleukin-1beta; Polymorphism, genetic; Craniocerebral trauma

INTRODUCTION

Severe traumatic brain injury (TBI) is the main cause of death in subjects between 1-45 years old. It generally involves some kind of sequela, with changes in consciousness, cognition or motor states, added to neural inflammation.[1] Each case outcome is related to several aspects, such as brain susceptibility, severity and extension of the injury, patient's age, other previous co-morbid factors, and also genetic aspects.[2]

Interleukin-1 is a cytokine which augments the inflammatory cascade by activating T cells, regulating the adhesion molecules manifestation and inducing other proinflammatory cytokines and associated proteins.[3] The interleukin-1 family consists of three related proteins which are encoded by genes in the long arm of chromosome 2: IL-1α (encoded by the IL1A gene), IL-1β (encoded by the IL1B gene) and IL-1Ra (encoded

by the IL-1RN gene).[4] The agonists interleukin-1α and interleukin-1β share a very similar tertiary structure and regulation factors, affinities and functions.[5] IL-1A (IL-1Ra) receptor antagonist is a competitive IL-1α and IL-1β inhibitor.

IL-1β is encoded by a gene of which expression is controlled at transcriptional and post-transcriptional level and is strongly involved in the inflammatory response. This cytokine is produced by macrophages and astrocytes, and its levels are very increased after trauma.[6] There are indications that IL-1β is involved with neurodegenerative diseases, both acute and chronic, such as ischemia, seizures, multiple sclerosis in addition to Parkinson's and Alzheimer's diseases.[7,8]

Polymorphism of genes encoding IL-1 family proteins have been studied in several diseases due to its importance in inflammatory processes.[9] The -31C/T polymorphism results from replacement of a cytosine (C) for a thymine (T) in the -31 site of the IL-1B promoter region. This polymorphism is at an important gene IL-1B regulatory region (TATA-box) and significantly affects the DNA-proteins interactions in *in vitro* assays.[10] The T allele, both in homozygosis and in heterozygosis, is related to increased IL-1β production,[11] and may cause an exacerbated inflammatory response and worsened patient's status.[9]

This study aims to analyze the IL-1B gene -31C/T polymorphism role in severe traumatic brain injury patients, aiming to evaluate its influence on the primary early outcome (intensive care unit (ICU) discharge or death), and clinical variables correlation.

METHODS

Patients

A cohort of severe TBI patients staying in ICU was studied from August 2003 to October 2009, in three hospitals in the Porto Alegre's metropolitan region: Hospital Municipal de Pronto Socorro, Hospital Cristo Redentor (both in the city of Porto Alegre, RS, Brazil) and Hospital de Pronto Socorro Deputado Nelson Marchezan, in the city of Canoas, RS, Brazil. The inclusion criteria were: patients aged above 16 and below 70 years-old, male, with severe TBI (Glasgow coma scale, GCS: 3-8). The brain injuries could be either primary or secondary. Polytrauma was not an exclusion criterion. By the emergency department admission, the patients were initially evaluated, resuscitated and went to emergency surgery, when needed. Only patients referred to the ICU up to 24 hours after the TBI were included in the study. All patients were sedated and mechanically ventilated, and were not administrated corticosteroids. Patients' demographics and clinical data were collected from the patients' charts. The patients' clinical follow-up was performed daily until the primary outcome: ICU discharge or death. Previous studies have shown that there are relevant gender differences on the outcome pathophysiology following acute neurological injury[12] or systemic trauma.[13] A lower susceptibility to post-ischemic and post-traumatic injuries has been observed in women.[12,13] Thus, in order to avoid potential bias from gender-related aspects on post-TBI outcomes, only male subjects were included in the study.

This study was approved by the Universidade Luterana do Brasil's Ethics Committee, and had the agreement of the Hospitals Municipal de Pronto Socorro de Porto Alegre, Cristo Redentor and Pronto Socorro Deputado Nelson Marchezan. Due to the patients' unconsciousness, the informed consent was obtained from relatives who were informed about the study aims. Blood draws were only performed after the informed consent was obtained.

Genetic analysis

DNA was extracted from severe TBI patients blood samples using an non-enzymatic method.[14] The gene-of-interest (IL-1B) amplification was done by polymerase chain reaction (PCR) as described by Yang et al.[11] The amplification products were digested with *Alu*I enzyme at 37ºC for 12 hours. The fragments were checked by electrophoresis over 10% polyacrylamide gel for 90 minutes at 100 V. The gel was stained with silver nitrate.

Statistical analysis

Allele frequencies were determined by direct allele counting. Departures from Hardy-Weinberg equilibrium and frequency differences between groups were evaluated by the Chi-square test. The groups mean values were compared using the Mann-Withney's U test. All P values were two-sided, and a $P<0.05$ value was considered statistically significant.

RESULTS

This study included 69 severe traumatic brain injury men. Table 1 shows the study population clinical and demographic characteristics, stratified by outcome (ICU discharge or death). Severe TBI was associated

with 45% mortality. The mean patients' age was 34.8 years, which was similar for both groups. Survivors were admitted to the ICU with a GCS 5.9 ± 1.7, while those with outcome death had GCS 4.6 ± 1.6 (P<0.01).

In the total patient's sample, 31 (45%) underwent craniotomy, with a significantly increased craniotomy frequency among the dead patients (P<0.05). The ICU stay time ranged from less than 1 day up to 54 days, with a significant difference when discharged patients (16.8 ± 13.8 days) were compared to the dead patients (3.7 ± 3.6 days; P<0.001). The Glasgow outcome scale (GOS) had also a significant difference between patients when stratified by outcome (survivors 3.4 ± 1.1; dead 1.0 ± 0.0; P<0.001). The main injury cause was related to traffic accidents (45% automotive accidents and 20% auto-pedestrian accidents). It was found that 42 patients (61%) had polytrauma associated to TBI, involving mainly thorax and limb injuries.

The allele and genotype frequencies in this population are shown in Table 2. In the total patients' sample, alleles C and T frequencies were 67% and 33%, respectively. No significant differences were found in allele and genotype frequencies among surviving or not surviving patients. The clinical features comparison between homozygote CC patients and those with the T allele (CT and TT genotypes) showed no significant differences (data not shown), except for the craniotomy frequency, which was significantly higher among patients with the T allele (genotypes CT and TT: 60.0%; genotype CC: 29.4%; P<0.05).

Table 1 – Traumatic brain injury characteristics in the studied population, stratified by primary outcome (ICU discharge or death)

	All patients	Alive	Dead
Number of patients	69 (100.0)	38 (55.1)	31 (44.9)
Age	34.8 ± 13.1	33.1 ± 12.2	37.0 ± 14.1
Admission GCS **	5.4 ± 1.8	5.9 ± 1.7	4.6 ± 1.6
Systolic Pressure	122 ± 27	118 ± 24	126 ± 31
APACHE II**	14.8 ± 5.4	12.4 ± 4.5	18.0 ± 5.0
Accident types			
AMVA	31 (44.9)	21 (55.3)	10 (32.2)
Auto-pedestrian	14 (20.3)	8 (21.0)	6 (19.4)
Fall	12 (17.4)	6 (15.8)	6 (19.4)
Aggression	5 (7.2)	2 (5.3)	3 (9.6)
FAW	7 (10.2)	1 (2.6)	6 (19.4)
Craniotomy*	31 (44.9)	12 (31.6)	19 (61.3)
Days to the outcome***	11.0 ± 12.3	16.8 ± 13.8	3.7 ± 3.6
GOS at outcome***	2.0 ± 1.4	3.4 ± 1.1	1.0 ± 0.0
Polytrauma	42 (60.9)	25 (65.8)	17 (54.8)

GCS- Glasgow coma scale; APACHE II – Acute Physiologic and Chronic Health Evaluation II; AMVA – automotive vehicle accident; FAW- firearm wound; GOS - Glasgow outcome scale. Results expressed as N(%) or mean ± standard deviation. Comparison between survivors and dead patients : *P<0.05; **P<0.01; ***P<0.001.

Table 2 – Interleukin 1-beta -31C/T polymorphism allele and genotype frequencies in severe traumatic brain injury patients, stratified by primary outcome (ICU discharge/death)

	All Patients (N=69)	Alive (N=38)	Dead (N=31)	P value
Allele				
C	92 (66.7)	50 (65.8)	42 (67.7)	0.952
T	46 (33.3)	26 (34.2)	20 (32.3)	
Genotype				
CC	34 (49.3)	19 (50.0)	15 (48.4)	0.747
CT	24 (34.8)	12 (31.6)	12 (38.7)	
TT	11 (15.9)	7 (18.4)	4 (12.9)	

Results expressed as N(%).

DISCUSSION

There is considerable variability in the traumatic brain injury outcomes, and genetic aspects may influence both brain susceptibility to injury and the ability to neural renewal and reorganization. Thus, investigations on genetic polymorphisms may help understanding the determinant factors for patients' prognosis. Polymorphisms investigation in neuroinflammation related genes is interesting given the important role they play in TBI. The integration of patients' genotypes with clinical data may help establishing severe TBI prognostic factors.

Some studies tried to associate genetic features with outcome following severe TBI. Apolipoprotein E, one of the lipid-carrier proteins, is one of the most studied genetic aspects in TBI. Studies have shown a significant correlation between unfavorable post-TBI outcome and polymorphism on the apolipoprotein E gene.[15,16]

Among the molecules involved in the inflammatory response mediation following any skull or brain injury, cytokines, particularly interleukin-1, plays the major role. It is known that in stroke, Parkinson's, Alzheimer's or any disease associated with neurodegeneration there are increased IL-1 levels, mainly IL-1β. Low levels of IL-1β are expressed in healthy brains, however they are increased following TBI.[17] The amount of cytokine produced may be related to IL-1B gene polymorphisms. Thus, the inflammatory response to trauma may depend on the patient's genotype. The expression of genes involved in inflammatory response is controlled both at transcriptional and post-transcriptional levels. At the transcriptional level, promoter gene polymorphisms may result in differences on response to some pathological processes.

IL-1A, IL-1B and *IL-RN* genes polymorphisms were analyzed in studies aiming to identify their influence in TBI patients. A study by Uzan et al.[9] evidenced the existence of a genetic association between post-TBI outcome and *IL-1B* gene polymorphisms; 69 patients were studied, and two SNP (single nucleotide polymorphisms) evaluated: one at the -511 site and another at the +3953. It was found that patients with allele T in both sites had worse prognosis (death, vegetative status or severe sequelae), perhaps due to an addictive effect of these polymorphisms. Tanriverdi et al.[18] studied 71 TBI patients, and found no association between outcome and -889 *IL-1A* C/T polymorphism. However, this work was refuted by Wang et al.[19] due to possible methodological genotyping issues. Hadjigeorgiou et al.[20] analyzed the -511 C/T IL-1B polymorphism and variable number of tandem repeats (VNTR) polymorphism of IL-1RN gene and their association with hemorrhagic events in 151 TBI patients. They identified that when the *IL-1RN* gene had the T allele, the patients were more likely to have post-TBI hemorrhagic events. This allele was associated with increased IL-1Ra production. Johnson et al.[21] studied the influence of the *IL-1A* and *IL-1B* alleles on apoptosis, evaluating hippocampus samples from 38 patients who died following TBI. The authors found no correlation between the alleles and the measured amount of apoptosis.

As it can be observed, studies with IL-1 genes in TBI patients had different experimental designs, focused different genes, polymorphisms and TBI pathophysiology aspects, and thus, the results, although conflicting, are not comparable.

Previous studies have shown that *IL-1B* -511T/C and -31C/T polymorphisms are in complete linkage disequilibrium, i.e., the -511T allele is always found in association with -31C allele, and the -511C allele is always found in presence of -31T allele.[10,22] In the present study the -31C/T polymorphism was analyzed, and to the extent of our knowledge, there are no previous literature studies which analyzed this polymorphism regarding association with TBI. However, due to the linkage disequilibrium, the results of the present study can be compared to studies analyzing the -511 site polymorphism. Our results showed no evidence of significant association between -31C/T polymorphism and severe traumatic brain injury patients' outcome. It is important to highlight that the number of evaluated patients is not very much different from other TBI patient samples.

It is important to understand the role of cytokines and its polymorphisms on neural traumatic injury, in order to allow the development of most effective diagnostic and therapeutic tools in severe TBI. Understanding the cell mechanisms involved in neural injury will allow therapeutic evolution and rehabilitation strategies for severe TBI patients, thus reducing the TBI impact on public health.

CONCLUSION

Understanding the influence of genetic polymorphisms on TBI outcomes is just starting, with few studies so far published. Our results suggest that gene *IL-*

1B -31C/T polymorphism has no significant impact on severe traumatic brain injury patients' fatal outcome.

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RESUMO

Objetivo: O traumatismo crânio-encefálico é a principal causa de óbito em indivíduos com idade entre 1 a 45 anos. O desfecho do traumatismo crânio-encefálico pode estar relacionado, além de fatores pré-morbidade e gravidade do dano, com fatores genéticos. Genes que podem ter relação com o resultado pós-trauma vêm sendo estudados, porém, ainda existem poucas informações sobre a associação entre polimorfismos genéticos e o desfecho do traumatismo crânio-encefálico. O gene da interleucina-1 beta (*IL-1B*) é um dos genes estudados, pois esta citocina encontra-se em níveis elevados após o traumatismo crânio-encefálico e pode afetar de forma negativa seu desfecho. O objetivo do presente estudo foi analisar o polimorfismo -31C/T, localizado na região promotora do gene *IL-1B*, em pacientes com traumatismo crânio-encefálico grave visando correlacioná-lo com o desfecho primário precoce (alta do centro de terapia intensiva ou morte).

Métodos: Foram estudados 69 pacientes internados por traumatismo crânio-encefálico grave em três hospitais de Porto Alegre e região metropolitana. O polimorfismo foi analisado através da reação em cadeia da polimerase, seguida da digestão com enzima de restrição.

Resultados: O traumatismo crânio-encefálico grave foi associado a uma mortalidade de 45%. Não foram observadas diferenças significativas nas frequências alélicas e genotípicas entre os grupos de pacientes divididos pelo desfecho do traumatismo crânio-encefálico.

Conclusão: Nossos resultados sugerem que o polimorfismo -31C/T do gene *IL-1B* não tem impacto significativo no desfecho fatal dos pacientes com traumatismo crânio-encefálico grave.

Descritores: Interleucina-1beta; Polimorfismo genético; Traumatismos crâniocerebrais

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